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PHOTOSYNTHESIS

Melvin Calvin

June 1958

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Department of Chemistry and Radiation Laboratory
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Abstract

The use of tracer carbon, as carbon-14, has made possible considerable progress in the mapping of the routes taken by the carbon atom from CO_2 into plant substances. The techniques of separation and identification that have made this progress possible lie largely in the region of chromatography and radioautography involving fractional-gamma amounts of material. Most of the earlier steps of carbon incorporation are now known. In addition, a number of the later steps on the routes to amino acids and proteins and other plant substances are now under investigation.

As a result of the recognition of the earlier stages of carbon incorporation, a number of proposals have been made about the photochemical act itself. These proposals have led to the development of direct physical tests of their validity, and some results of these will be described.

The remaining principal area of investigation involving the route of oxygen atoms from water to molecular oxygen is largely unexplored, but the use of new methods of analyzing for the heavy isotopes of oxygen may make possible more progress in this area.

PHOTOSYNTHESIS†

Melvin Calvin

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June 1958

The problem of photosynthesis is the problem of defining the way in which green plants are able to convert electromagnetic energy into chemical potential in the form of reduced carbon, usually as carbohydrate, and molecular oxygen. Figure 1 will give you some idea of the place in which the tracer method found itself at the beginning of this work. This shows schematically the problem as we saw it in 1945. Light energy is absorbed by the chlorophyll in the green plant and in some way is converted into a form that leads ultimately to a reducing compound, here labeled H, and an intermediate oxidant between water and molecular oxygen, here labeled as A, B, and C. On the other hand, the reducing agent primarily produced (or even secondarily produced) by the light is used to reduce the carbon dioxide through a sequence of intermediates, here indicated by X, Y, and Z, ultimately bringing the carbon to the level of carbohydrate. This was approximately the state of our knowledge in 1945 when the cessation of the war permitted a resumption of activities in this direction. Perhaps the strongest stimulus to this work was the availability of carbon-14, which has a 5000-year half life. Large amounts of carbon-14, relatively cheaply made in nuclear reactors, really provided the enormous stimulus for tracing the path (shown in Fig. 1) that carbon atoms take from carbon dioxide near the bottom, through whatever sequence of compounds it may appear in to the carbohydrate at the top. And here we began our work in January of 1946.

Figure 2 illustrates diagrammatically the tracer method used in the path-of-carbon studies.[1] The leaf represents the green plant that we use, and it illustrates the principle of the tracer method. The carbon dioxide enters the leaf in the form of the CO_2 stream that the leaf is absorbing, and it passes through whatever intermediates may be involved, marked X, Y, and Q, ultimately ending up as sugar. Other compounds present in the plant do not immediately acquire radioactive carbon because they are not directly on the tracer line. This is, in essence, the principle of the method that we use. Of course, if we let the radioactive carbon be absorbed by the plant for a long period of time, it will find its way into sugar. If we cut the time down, we will find only X and Y; if we cut it still shorter, we will find only X. Compounds not on the path, such as Q, will not acquire the label.

In order to do this kind of work we had to have a reproducible living organism, an organism that would perform at the request of the chemist and give the same answer every time a specific question was put. This wasn't easy to devise--for a chemist--so we had to devise our own "farm" on which we could grow the organisms we would use for this study in such a way that we could depend upon their identical behavior from day to day. Our present farm consists of a continuous tube culture of unicellular green algae whose density is controlled by a photocell. Figure 3 shows a photomicrograph of the algae themselves. These are Chlorella. Here you have some idea of the size. The cup-shaped chloroplastis, with the cytoplasm around them, show very well near the center.

†The work described in this paper was sponsored by the U.S. Atomic Energy Commission.

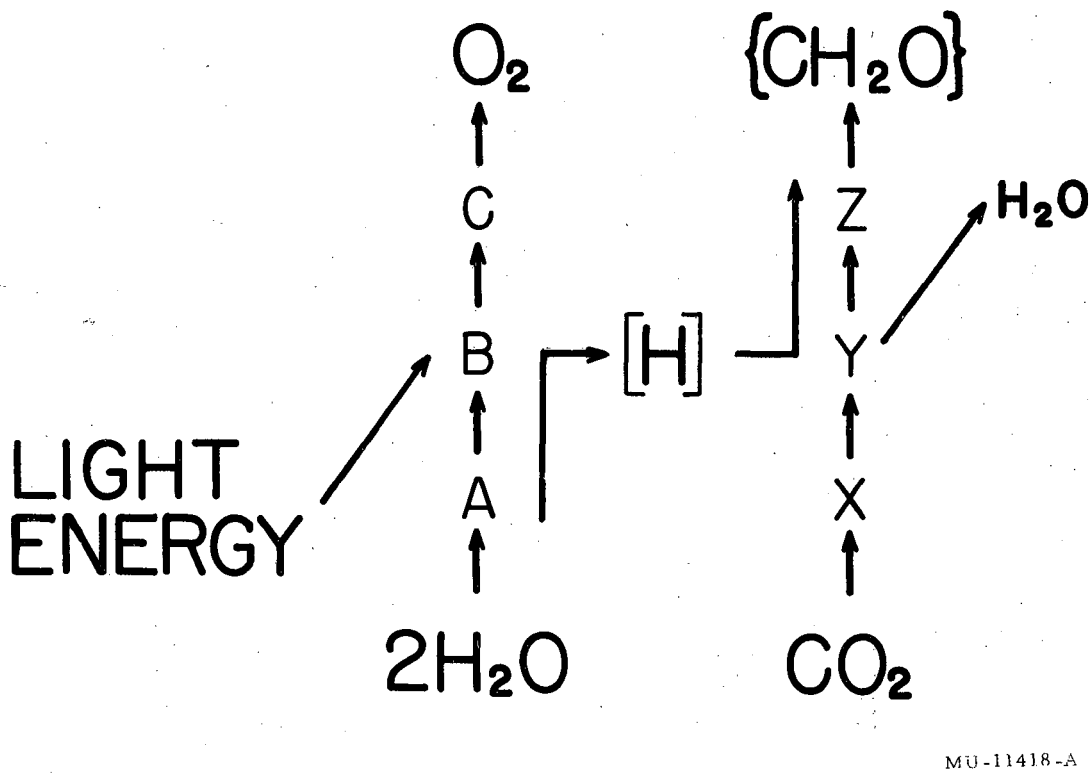

Fig. 1. Elementary photosynthesis scheme.

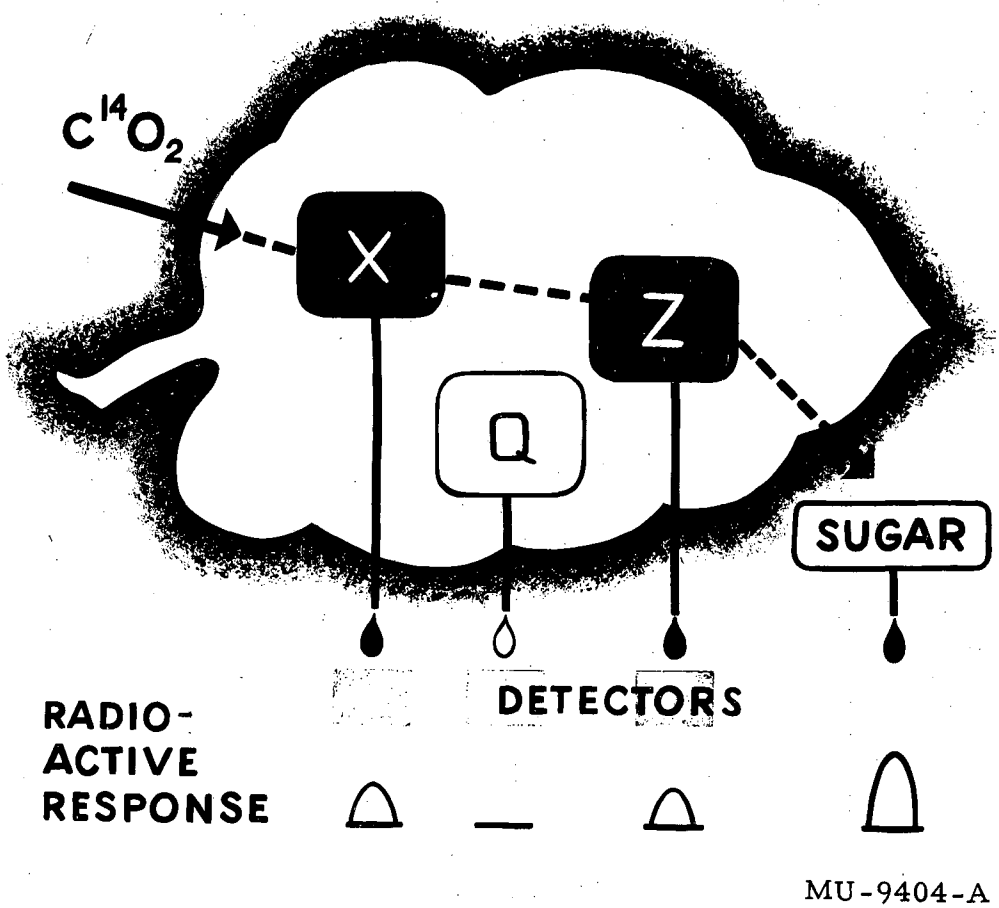

Fig. 2. Schematic representation of C^{14} labeling of reduction products in the leaf.

Having devised the farm and grown the algae, we then expose the algae to radiocarbon for a short period of time. Following the exposure, the algae are killed in a variety of ways, and an extract of the algae is prepared which will be used for analysis. The method of analysis that we use is paper chromatography.[2] A small bit of the extract is placed on a corner of a piece of filter paper, suitable solvents are allowed to run over the paper, thus separating the mixture of compounds into its various constituents, and then we find which ones are radioactive by exposing a photographic film to the paper. Wherever there is a radioactive compound on the paper the film becomes black, and we thus know where the radioactive compounds are on the paper. Then we proceed to determine what they are. From their position on the paper, we have a clue as to what they are, but not a complete identification. Having found them, we can then elute them off the paper and perform chemistry, put them back on the paper again, and see what happens to them. In this way, we were ultimately able to identify the compounds.

As a result of this operation, we have, in Fig. 4, a chromatogram showing th spots. This shows you what happens after 30 seconds' exposure to radiocarbon, and you will see that there are about a dozen compounds here that are labeled (there are a lot more that are not labeled). Thirty seconds, you see, is much too long, and so we shortened the time; Fig. 5 shows a chromatogram of a 5-second exposure, and you can see one compound predominating. It requires a much more detailed kinetic analysis to determine which might be next in line. As far as our isolation procedure is concerned--namely, hot alcohol and extraction--3-phosphoglycerate is the first one we can isolate by this method and identify. Other methods of isolation have since been tried, and we have found what we think may be predecessors to phosphoglycerate as well.

The phosphoglycerate has three carbon atoms in it; thus it became necessary for us to determine which of these three carbon atoms has the radioactive carbon in it, and if it were in more than one of these carbon atoms, in what ratios. This involved degradation, and we found that at the very shortest times the carboxyl group of the phosphoglyceric acid, whose structure is shown in Fig. 6, was the one that was labeled. Now the phosphoglyceric acid (PGA) is in the upper left-hand corner. The phosphorus with a circle around it is a phosphoric acid ester group (PO_3H_2), and the star indicates the atom that contains the greatest amount of radioactive carbon. Following this, from the same experiment, we were able to isolate a six-carbon sugar, fructose or glucose, represented in Fig. 6 by fructose diphosphate (FDP). We also took apart this six-carbon fructose molecule and found that the label was largely on the two carbon atoms in the middle. This immediately calls to mind the way in which hexoses are split, that is, they are taken apart by splitting a hexose in the middle, making two three-carbon pieces, and then on down. We then suggested, six or eight years ago, that the hexose was made by the reverse process, from the three-carbon phosphoglyceric acid through the triose, ultimately giving the hexose. It gave us a fairly clear picture of how the six-carbon unit was put together from three-, but it gave us no information about the origin of the three-carbon piece itself except that the carboxyl group of the phosphoglyceric acid was clearly the one that was coming directly from the CO_2.

Here is where our arithmetic deceived us a little bit. Because the carboxyl group comes from the carbon dioxide, which is a one-carbon compound, and the resulting compound is a three-carbon compound, we reasoned that we must look for a two-carbon compound to make up the difference. This is where we made a mistake. It was a good enough reason at the time, and it led us not to a two-carbon compound

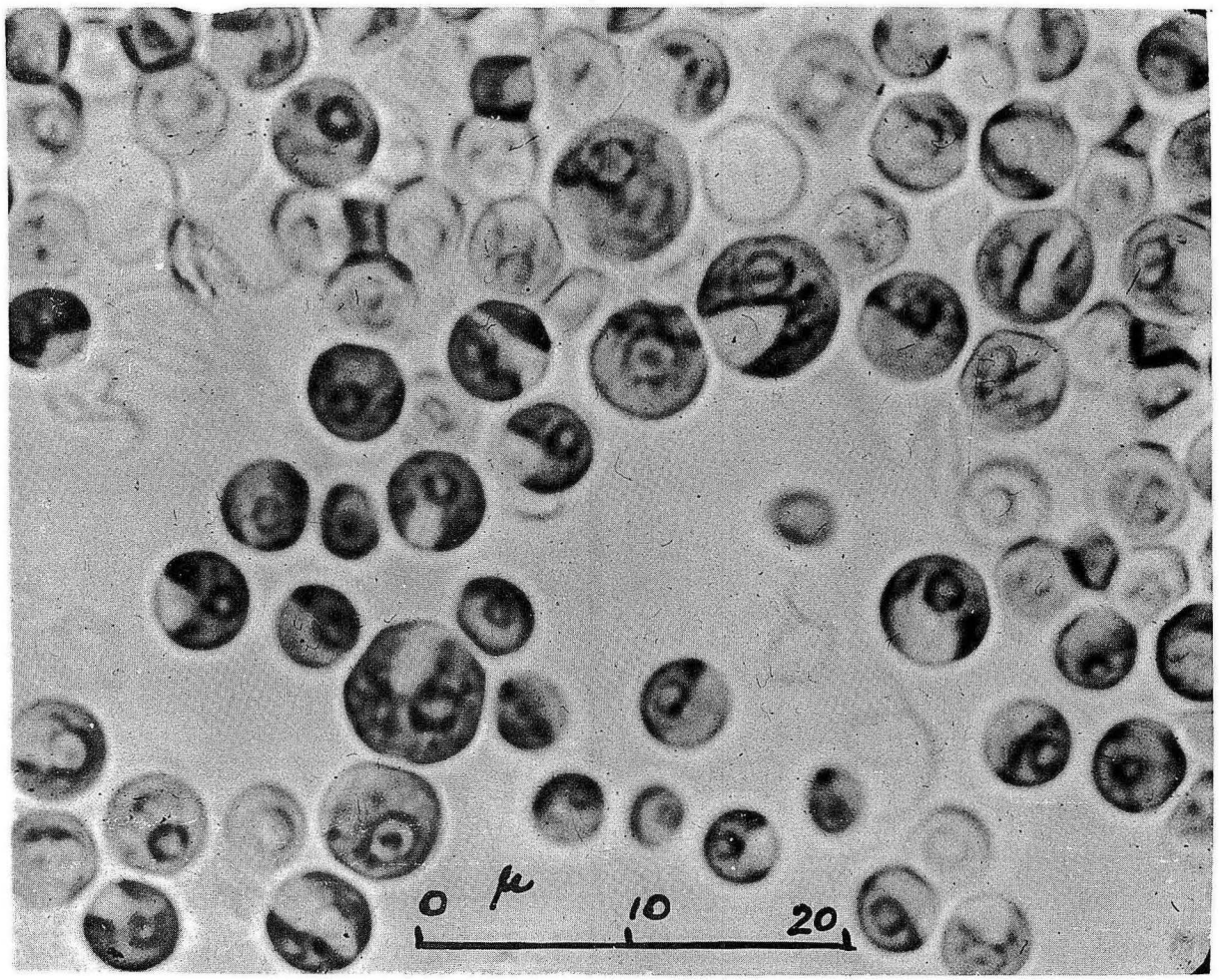

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Fig. 3. Photomicrograph of Chlorella.

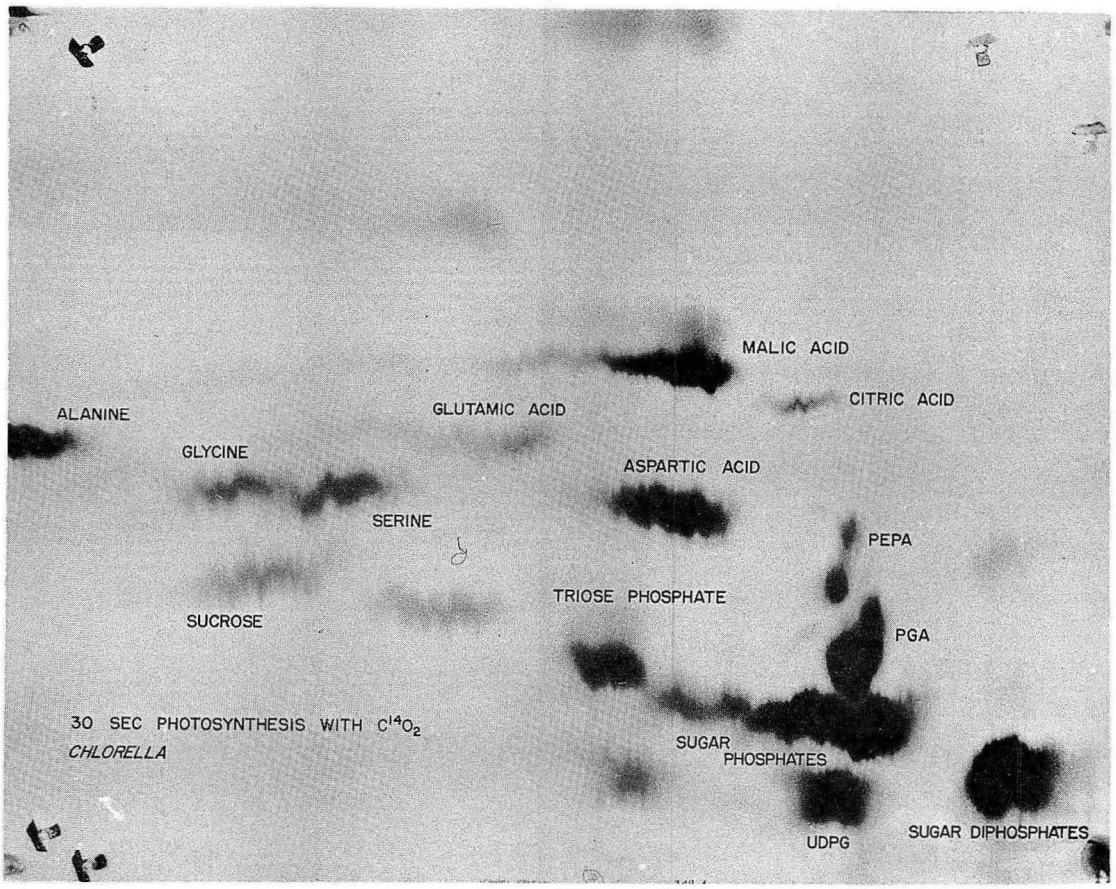


Fig. 4. Chromatogram of extract from algae indicating uptake of radiocarbon during photosynthesis (30 seconds).

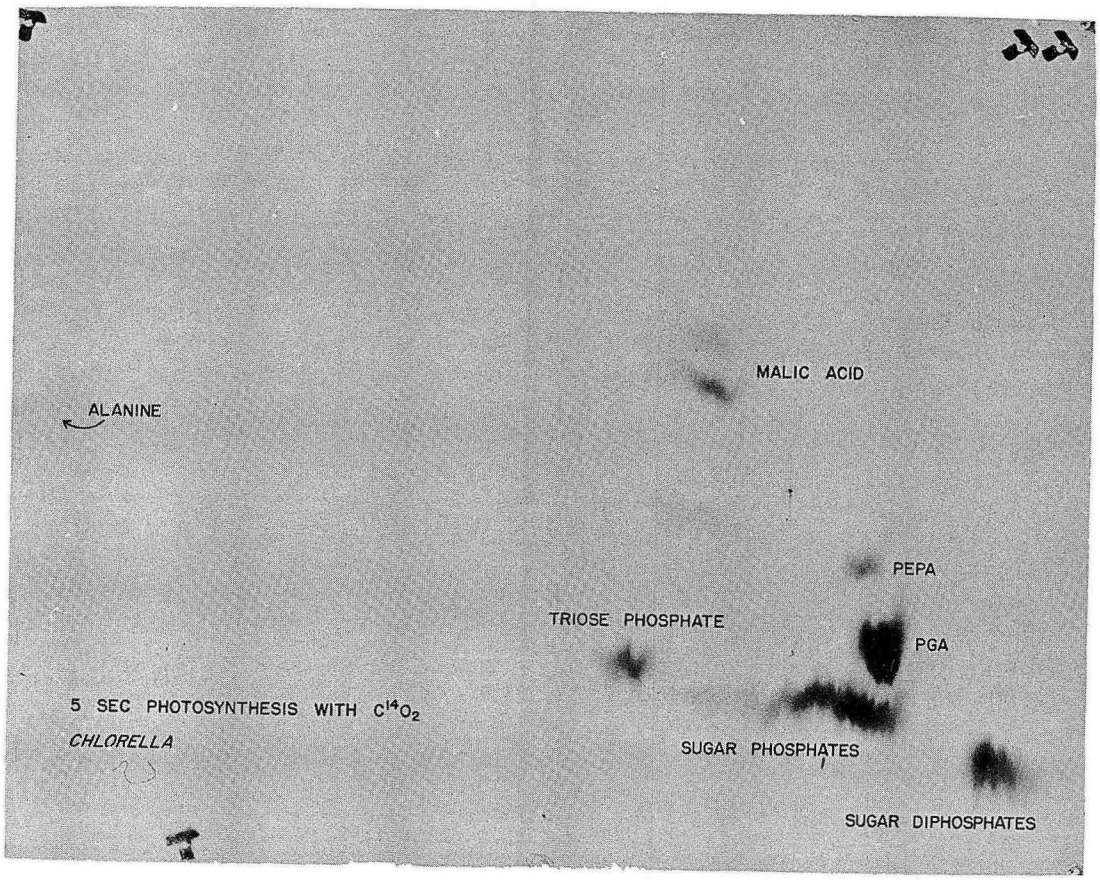


Fig. 5. Chromatogram of extract from algae indicating uptake of radiocarbon during photosynthesis (5 seconds).

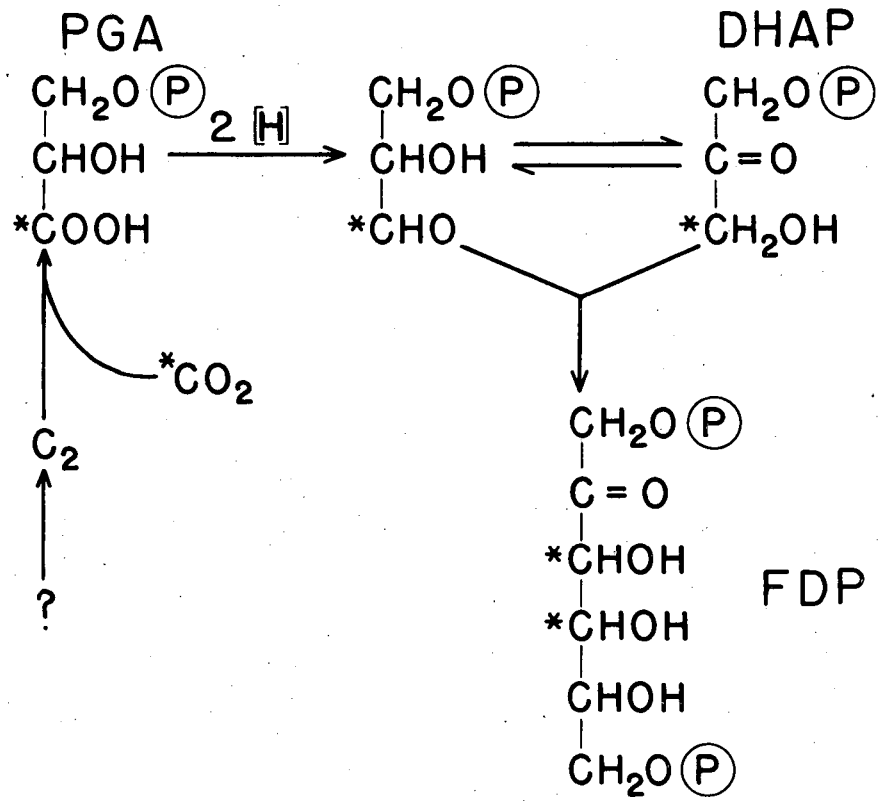


Fig. 6. Path of carbon from CO_2 to hexose during photosynthesis.

but to almost everything else but a two-carbon compound. We found a five-carbon sugar, but we also found a seven-carbon sugar, which was very strange. Figure 7 shows the names and formulas of these compounds, together with the distribution of radioactivity in the carbon atoms of each. Here on the left are shown phosphoglyceric acid (PGA), the five-carbon sugar (or a representative of it) labeled RuDP, ribulose diphosphate; in the middle, the seven-carbon sugar labeled SMP, called sedoheptulose monophosphate; and finally a skeleton representation of the six-carbon sugars on the right-hand edge of the figure. The stars are placed to give some idea of the relative distribution of the radioactive carbon in each of these compounds. You will notice that the pentose has a very queer distribution of radioactive carbon. The two top carbon atoms of the RuDP have activity in them (a small amount), the middle carbon atom is very "hot," and the bottom two have very little activity. You will find in looking at this sequence that the five-carbon distribution does not exist intact in either the six- or the seven-carbon compounds, and we had, therefore, to devise some way of creating the five-carbon compound that has this particular distribution. It was not until we realized that the five-carbon sugar, ribulose diphosphate, had more than one origin that we were able to devise a way of creating a five-carbon chain with that particular distribution of radioactivity. We did it in the manner shown in Fig. 8.

Here you see two five-carbon sugars, xylulose monophosphate and ribose monophosphate, created by taking two carbon atoms off the top of the sedoheptulose and adding them to a triose phosphate. When you take two off a seven, a five is left; that is the ribose. When you add two to a three, a five is created; that is the xylulose. What we were analyzing was ribulose made from both of these, and this, then, when the distribution is averaged is what we needed. We manufactured it from three- and seven-carbon molecules. The three-carbon triose, we make from the phosphoglyceric acid. We make the five- by a combination of a seven- and a three-carbon molecule. Now we have to devise a way of making the seven with that particular curious distribution. This became possible when we realized that the seven-carbon piece could be split between C_3 and C_4 by a reaction almost exactly the same as the reaction by which the hexose was made. This suggested that the seven-carbon sugar was made from a four- and a three-carbon piece. Now the three we have already, so that we have only to get a four with the proper distribution. A four of this distribution exists in the hexose, and this is shown in Fig. 9. Here you see the triose--that is, two trioses in equilibrium with each other, one a ketone and the other an aldehyde, one of them reacting with the hexose to form another pentose and the tetrose sugar phosphate with the labeling we need. The tetrose then combines with this other triose to form a heptose, the seven-carbon sugar with the proper label. Thus we have created the heptose, the hexose, and the pentose and triose that we needed.

From all this, we have not yet found how the triose precursor, namely, phosphoglyceric acid, is created. This was not possible for us until we made quite a different kind of analysis, an analysis of the kinetics of the rise and fall of the compounds in the plant as a function of changes in the external environment. The biggest change that we can make in the external environment of the plant is to turn the light off, since this is the ultimate source of energy. Figure 10 shows what happens when that is done. This shows you a method of using the amount of radioactivity that one finds on a piece of paper to determine how much of a particular compound is present in the plant. With the light on and the plant photosynthesizing in radiocarbon for a long period of time you see that the phosphoglycerate spot on

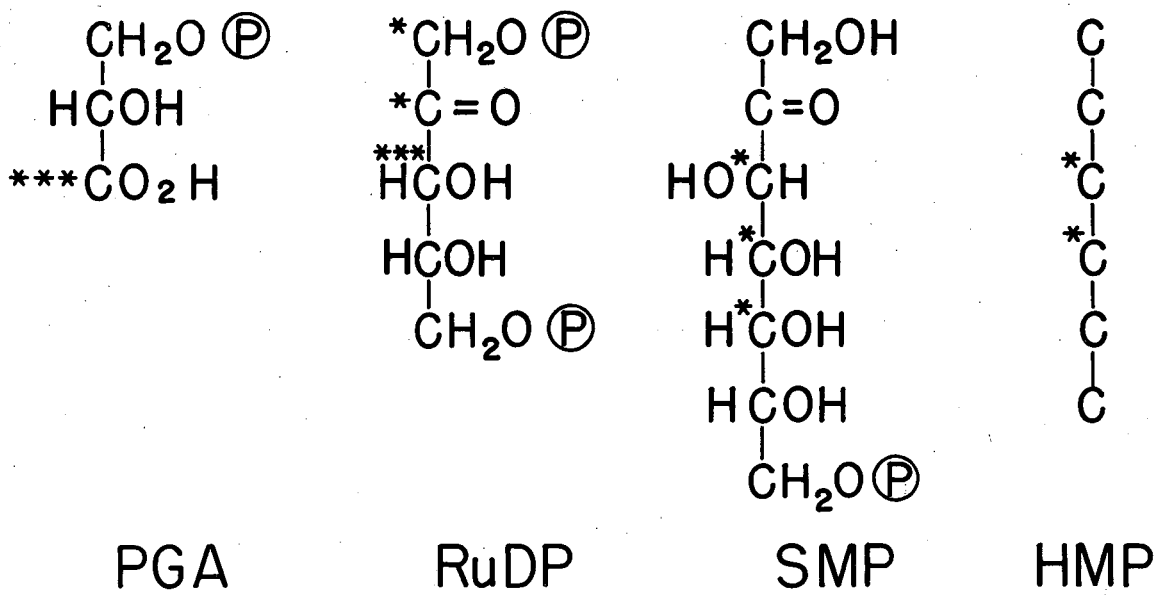


Fig. 7. Distribution of radioactive carbon in certain sugars.

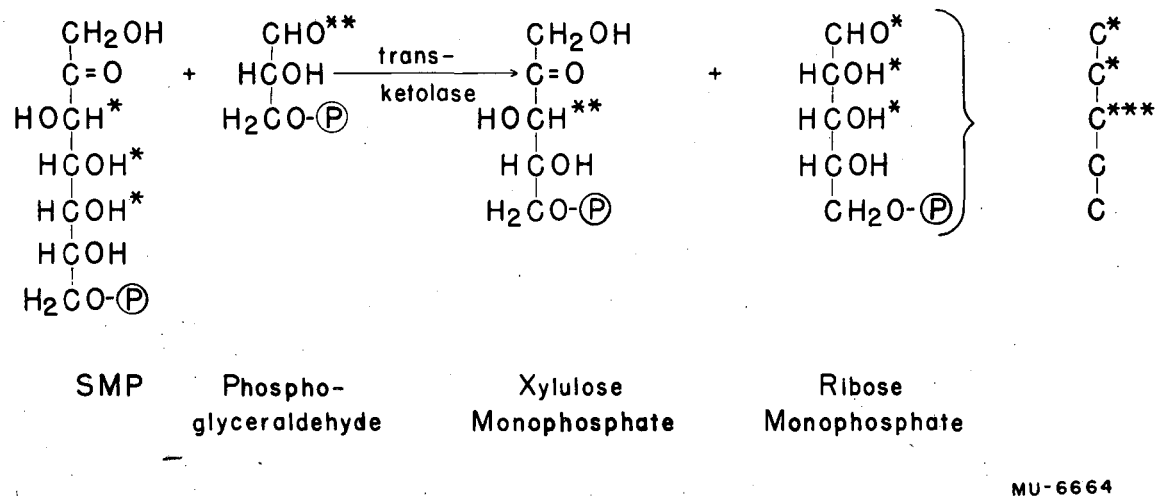


Fig. 8. Proposed scheme for labeling of pentose.

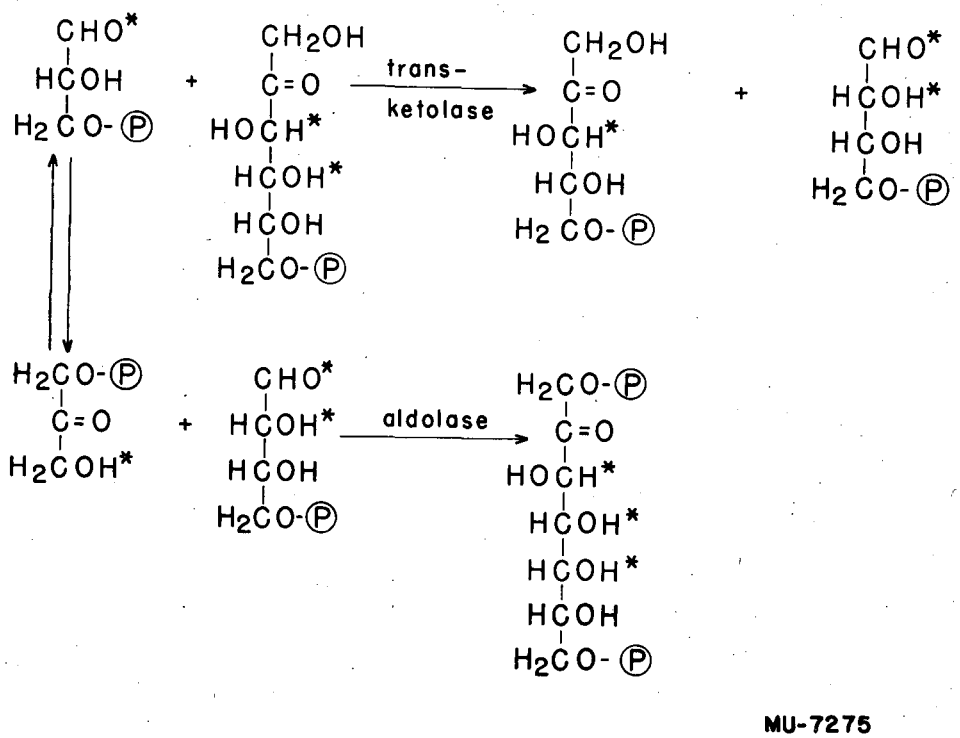


Fig. 9. Formation of a heptose from triose and hexose.

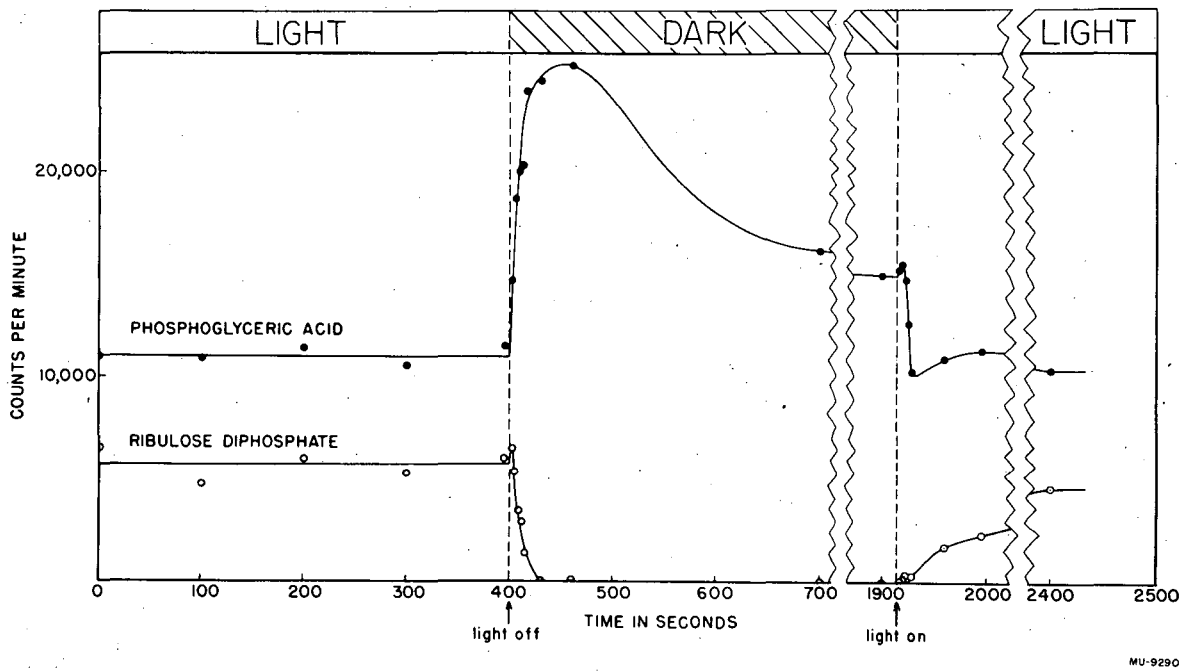


Fig. 10. Light-dark changes in concentrations of PGA and RuDP.

the paper is pretty much constant. In other words, we have saturated the PGA with radiocarbon, and we can make a count on the PGA spot and find out from this and the specific activity of the radiocarbon that is fed into it how much PGA there is in the plant. The same thing is true for ribulose diphosphate. At 400 seconds on Fig. 10 the light is turned off. Now we begin to see a rapid transient change. From an analysis of these transients, we were able to take our next step. You will notice that what happens here as soon as the light is turned out is that the PGA rises steeply and the RuDP falls sharply. This antithesis of behavior immediately suggested that the one was going into the other--that the ribulose was going into the phosphoglycerate--and this helped us to find the origin of the three-carbon piece. Ribulose diphosphate is a five-carbon compound, and we add CO_2 to that and get six carbon atoms, which can make two molecules of the three-carbon piece, phosphoglyceric acid.

Now from this we were led to a simplified cyclic scheme, which is shown in Fig. 11. The RuDP in the upper left is the ribulose diphosphate again, adding CO_2 to make PGA, the three-carbon piece. From this, using light energy, we make the triose phosphate; that is, we reduce the three-carbon acid to the sugar level, and then by a series of sugar rearrangements which have been shown earlier, the three-carbon sugar goes to the six- and the seven-, ultimately coming back to the five-carbon sugar, ribulose monophosphate, shown in the lower left. Then another phosphorus is added, as a result of the light action, to make the RuDP, and the cycle is complete. This cycle was suggested as a result of the experiment on transients in Fig. 10.

But it, in turn, suggests another experiment that had not yet been done, as far as we were concerned, namely, on the transient resulting from the change in the concentration of the CO_2.[3] Just as we turned off the light and blocked the step between PGA and triose, so, presumably, we have control from the outside of the step between ribulose diphosphate and PGA in terms of the CO_2 level. We can, for example, suddenly remove the CO_2. If we do that, we may expect that the amount of the RuDP will rise because it has no CO_2 to react with it, and the PGA will fall because the light is removing it and it is not being formed in the carboxylation reaction. This experiment is much more difficult than the light-dark one, but it has been done. The transients have been analyzed, and the results are shown in Fig. 12. Here you see the cycle shown diagrammatically; on the left we are running at 1% CO_2, and at the line labeled zero time the stopcock is turned and we change from 1% CO_2 to 0.003% CO_2. You can see that the initial prediction is amply fulfilled; PGA falls very rapidly here while the RuDP is rising very sharply.

Having identified ribulose diphosphate as the carbon dioxide acceptor,[4] we became interested in finding out if we could do this reaction outside the plant, since all the others had been done outside the plant by this time. For this we need a catalyst, as well as the RuDP. The catalyst would be an enzyme system presumably present somewhere in the plant, again presumably very closely associated with the light reaction (with the light-catching apparatus in the chloroplasts). We isolated some chloroplasts and washed them with water; the enzyme washes right out of the chloroplasts very easily, resulting in a fairly clean preparation of the enzyme that catalyzes the carboxylation of RuDP all the way to phosphoglyceric acid.[5,6] This reaction is shown in Fig. 13. Here the RuDP reacts with the CO

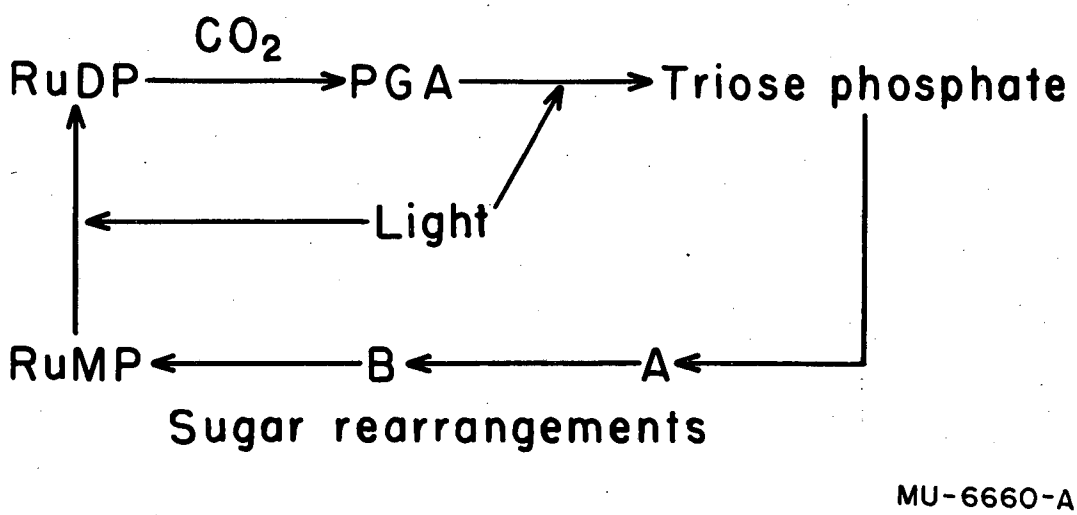


Fig. 11. Formation of five-carbon sugars from ribulose diphosphate.

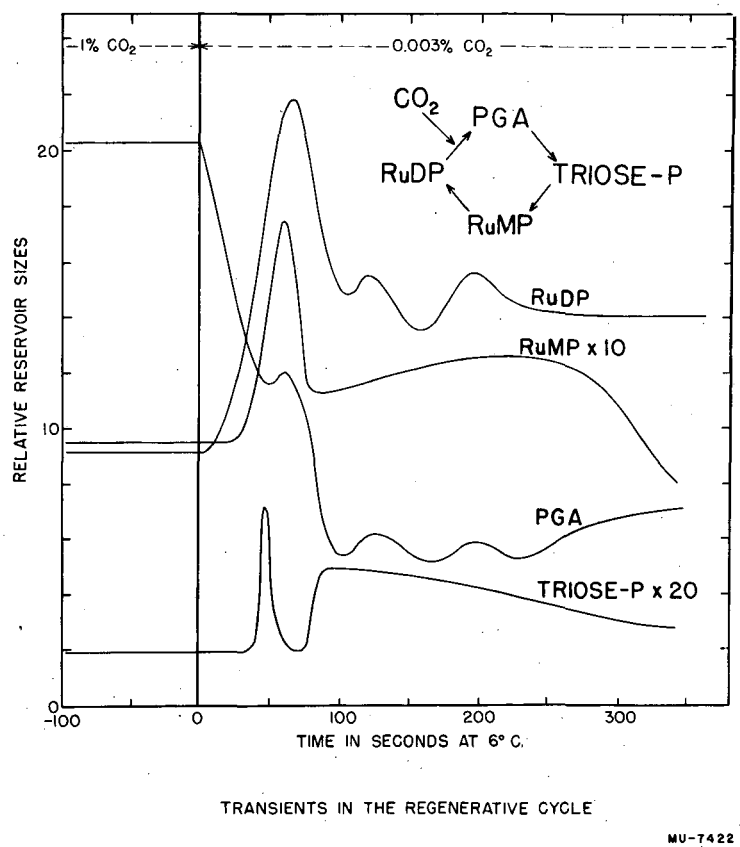


Fig. 12. Transients in the regenerative cycle.

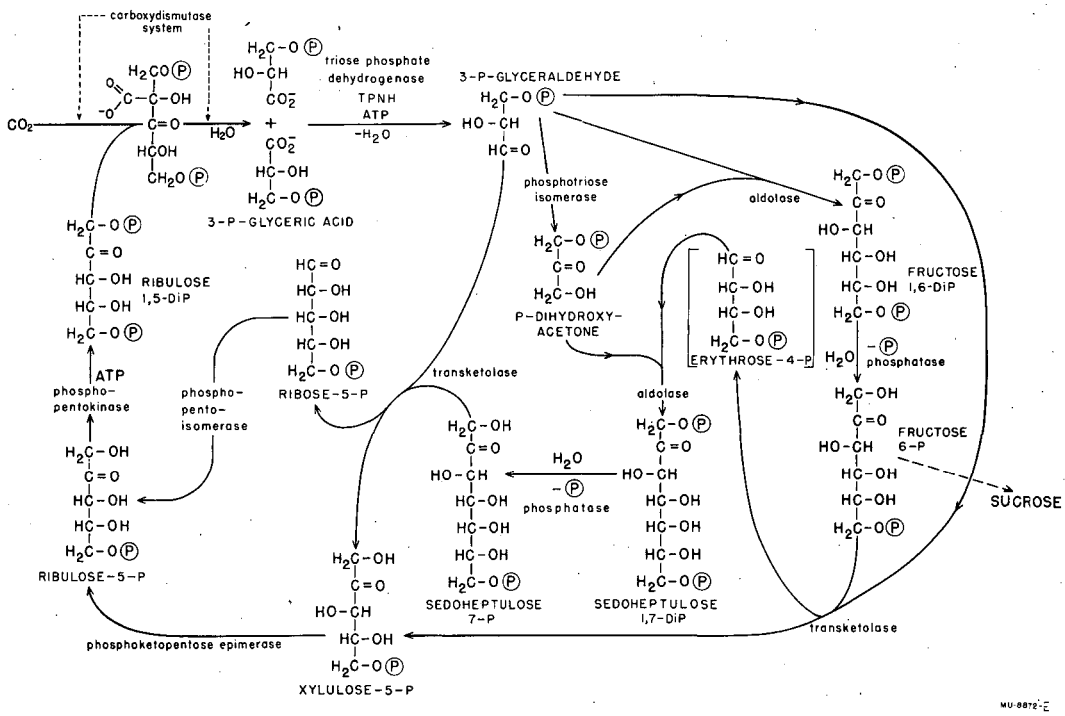


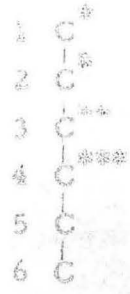
Fig. 13. The photosynthetic carbon cycle.

coming up to it. Although this may appear as a proposal of mechanism, it is written thus to indicate intermediates. The RuDP will react with CO_2, or something very closely related to CO_2, to form a keto acid intermediate. This keto acid may be immediately hydrolyzed by water, to give two molecules of PGA. Now this reaction results in a rearrangement of the redox levels of the various acids involved. The CO_2 starts out much more oxidized, and it ends up as a carboxyl group, a bit reduced. In order to do this, something else must be oxidized, and the thing that is oxidized, of course, is the No. 3 carbon atom of the ribulose, which goes from an enol, alcohol, or ketone to a carboxylic acid level. Therefore, we call this whole system carboxydismutase, meaning a carboxylation and dismutation of redox levels, one going up and the other going down.

We have evidence now that the intermediate keto acid actually exists.[7] We have seen it in the diphosphate area of Fig. 4. There is also an indication of an intermediate lying between CO_2 and the keto acid. The evidence is in the form of very labile radioactivity. It behaves very much as though it were a mixed anhydride of adenylic acid and CO_2, which might be enzyme-bound.[8,9,10] There seem to be two things, now, between CO_2 and PGA. These are the labile complex that I mentioned and the keto acid, 2-carboxy-3-ketopentose-1,5-diphosphate. While the latter, and a close relative of it--namely, 2-carboxy-4-ketopentose-1,5-diphosphate--have indeed been seen,[7] the evidence for the labile, or "active," CO_2 which would precede this is much less certain. This exists primarily in the form of an additional total fixation, determinable by other than plating methods when the algae are killed in cold organic solvents. No other direct evidence of this labile, or "active," CO_2 is yet available to us.[10] In some of the early experiments we believed that we had trapped the labile CO_2 by treatment with hydroxylamine at low temperatures followed by chromatography. However, the spots appearing in these hydroxylamine-treated chromatograms have since been shown to be chromatographic artifacts due to the presence of hydroxylamine.[11]

Figure 13, which shows the photosynthetic carbon cycle as it now stands in all its details, contains in it one compound enclosed in brackets. This is erythrose-4-phosphate. Although both the formation of erythrose-4-phosphate from 3-phosphoglyceraldehyde and fructose-6-phosphate, and its combination with dihydroxyacetone phosphate to form sedoheptulose-1,7-diphosphate have been demonstrated in vitro,[12,13,14] erythrose-4-phosphate has not, until now, been detected in extracts of algae allowed to carry on photosynthesis in the presence of $C^{14}O_2$, nor, as far as we are aware, has it been found to occur naturally in any other plant system. We have recently been able to demonstrate the presence of erythrose-4-phosphate amongst the sugar phosphates in Chlorella that were incubated in the light for 3 minutes in the presence of $C^{14}O_2$.[15] This was possible because of the extremely long development times that were used to separate the phosphate areas in both chromatographic dimensions. Many new phosphate-containing spots appeared under these circumstances, and among them was found one which, after dephosphorylation with phsophatase, produced erythrose. It thus appears that every stage of the photosynthetic carbon cycle shown in Fig. 13 is now known and has been carried out enzymatically as a separate reaction, and every compound shown has been demonstrated to be present in the photosynthesizing algae.

In recent months there have appeared a number of hexose degradations in a variety of photosynthesizing sources, having the general pattern indicated in the following diagram.[16, 17]



This peculiar distribution led Gibbs and Kandler to reject the possibility that hexose could be made by the condensation of phosphoaldotriose with phosphoketotriose. An examination of the one published chromatogram[16] showsthat ribulose diphosphate and hexose monophosphate radioactivity dominated a 1-minute photosynthesis from Chlorella, producing hexose labeled in this way. This has been accounted for[18] in terms of a feedback, via transketolase, of the top two carbon atoms from the pool of ribulose onto the lower four carbon atoms of the pool of sedoheptulose, to produce the kind of hexose labeling here seen. The lower value of the activity of C_3 relative to C_4 is accounted for by a slight lag in the equilibration between aldotriose and ketotriose. The presence of a large pool of diglycerolphosphate to act as a diluent for the ketotriose would accentuate this effect.[19, 20]

Another apparent anomaly was described by Kandler[21] in connection with the lack of ability of the plant to produce sufficient adenosine triphosphate (ATP) to run such a cycle. This lack of ability was determined through the use of cyanide inhibition experiments. An attempt to study the pattern of CO_2 fixation in the presence of cyanide led to the isolation of an unknown acid diphospate by Kandler.[22-24] This diphosphate was identified as the diphosphate of a close relative of hamamelonic acid by Rabin et al.[25]

On the basis of this evidence it was suggested that the intermediate keto acid shown in Fig. 13 was actually reduced photochemically to form hamamelonic acid diphosphate. This was then thought to be reduced further and rearranged to form a hexose without splitting to give three-carbon fragments. Whether or not this is possible remains yet to be determined. None of the several possible stable intermediates required in such a scheme has yet been seen in any tracer experiment.

However, the last evidence adduced for it was shown to be in error by Rabin's discovery that the hamamelonic acid was not a result of the carboxylation reaction but rather was the result of cyanhydrin production from the ribulose diphosphate followed by hydrolysis to produce the hamamelonic acid diphosphate (or its close stereochemical relative).[25] This was further confirmed by showing that KCN would add to ribulose diphosphate in vitro to give the same result. And, finally, by the use of radioactive cyanide and unlabeled carbon dioxide, the formation of hamamelonic acid diphosphate in the green plant under circumstances identical to those of the previous experiment was demonstrated.[25] It thus appears that the experiments dependent upon the concept that cyanide inhibits primarily the electron-transport system and the production of ATP must be revised, since it clearly will react very rapidly with at least certain carbonyl derivatives in the plant viz., ribulose diphosphate.

Still another experiment of the same sort has been described in which cyanide is reported to obliterate the formation of glyceric acid.[26] This is, of course, what one would expect if the cyanide reacted with all of the carbon dioxide acceptor (ribulose diphosphate) so that the glyceric acid could not be re-formed, while the photochemical reaction reduces whatever glyceric acid--and its derivatives--may already be present to sugar.

Another experiment which seems to have a bearing on this problem was recently reported by Gibbs, who fed labeled formate, in the presence of 5% unlabeled CO_2, to algae.[27] From these algae he was able to isolate starch from which glucose containing most, or all, of its label in the C_4 position was obtained. This was said by Gibbs to indicate that CO_2 (at the 5% level) inhibited the photosynthetic carbon cycle and that formate was an intermediate on the way between carbon dioxide and hexose. Since algae are quite capable of growing (and growing well) in 5% CO_2, it seems more reasonable to suppose that the formate is entering the hexose through some route quite independent of the photosynthetic carbon cycle--for example, by addition of glycine to form serine and then around into the hexose--and that this hexose is quite separate from that participating in the photosynthetic carbon cycle.

Still another reaction that occurs in plants is the one observed by Warburg which leads to the formation of α-labeled glutamic acid via the carboxylation of γ-aminobutyric acid.[28] That this reaction occurs has been amply demonstrated in many cases, particularly by Fuller,[29] and Warburg's observation that fluoride in some way induced the decarboxylation of glutamic acid to carbon dioxide and γ-aminobutyric acid seems well established.[29, 30] This seems to have little to do with the photosynthetic carbon cycle. However, it is conceivable that this source of a one-carbon compound might serve some function in buffering the carboxylation of the photosynthetic carbon cycle.

That other carboxylation reactions can take place in photosynthetic organisms has long been recognized;[31, 32, 33, 34] for example, the increased rate of malic and aspartic acid formation in the light was recognized in our very earliest experiments. This we attributed to the increased level of the three-carbon acceptor, such as phosphopyruvic acid or pyruvic acid itself, which could lead to aspartic acid. A similar reaction has been demonstrated in the purple bacteria by Fuller.[35] Undoubtedly there are other points of entry of one-carbon compounds into the carbon skeletons in green plants as well as other organisms. That these points of entry will be affected by light may very well be expected in view of the fact that the intermediates required for the reactions with carbon dioxide must be drawn from the photosynthetic carbon cycle as shown in Fig. 13. Increased concentrations of reduced cofactors from the light reaction might also be expected to stimulate reductive reactions (e.g., malic dehydrogenase).

Figure 14 shows the relation of the carbon-reduction cycle in photosynthesis to the photochemistry involved--that is, to the radiation chemistry with which, after all, we are ultimately concerned. The machinery--the gearing--that runs the cycle comes in on the upper left. First of all we have the quantum entering, accepted by the chlorophyll and transformed in some way to give a reducing agent, here labeled $[H]$ and an oxidizing agent, here labeled $[O]$. The $[O]$ ultimately becomes molecular oxygen. The $[H]$ is used to reduce the glyceric acid to triose.

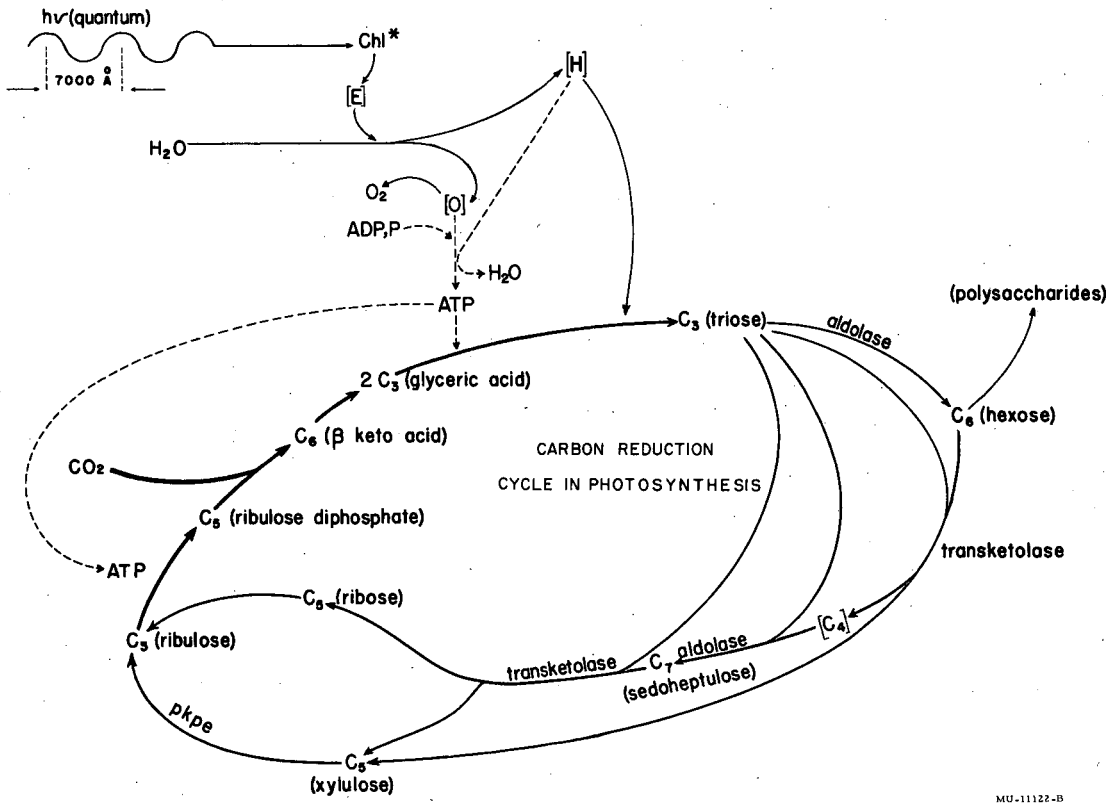


Fig. 14. Suggested cyclic scheme for relationships in photosynthesis.

Here we must be concerned with the remainder of the process, and the next thing we want to discuss is the path of oxygen. You will notice what happened to the path of carbon after some ten years of investigation. From CO_2 on one end to polysaccharides on the other, we have the whole scheme of cyclic intermediates. These were all pretty much worked out by use of the radioactive methods of analysis already described. Unfortunately, we don't have a radioactive isotope of oxygen that lives long enough to give us the time to do this. The only thing we know about that path of oxygen is due to the work of Ruben, my predecessor, who was able to show--using heavy oxygen (oxygen-18)--that the molecular oxygen from the plant has its primary source in the water molecule and not in the CO_2. The oxygen atoms of the CO_2 have to go by exchange into water before they come out as molecular oxygen.

The reason that Ruben was able to do this twenty years ago was that he knew what the beginning of the reaction was, namely, water and CO_2, and he knew what the end was, molecular oxygen. Thus he could determine the relation between the two with the O^{18} and the mass spectrometer. However, he could not, using these methods, determine what went on between these two extremes, since in order to make an analysis in the mass spectrometer it is necessary to isolate the material and it is precisely this thing which one could not do without knowing what to isolate.

A method has been developed that will permit us to do very much the same sort of thing with oxygen-18 as we have done with carbon.[36] This method depends upon the fact that oxygen-18 can be activated by proton bombardment rather specifically to fluorine-18. F^{18} is radioactive, with a half-life of about 2 hours. What we do is to perform an ordinary photosynthetic experiment, but instead of using ordinary water we used heavy water, H_2O^{18}. Then we make a chromatogram as before; we can only do one-dimensional chromatography. In order to determine where the O^{18} is on the chromatogram, we hold it up in front of the cyclotron and spray protons at it. The activated chromatogram is allowed to cool for a few minutes and then we take a picture of it, a radioautograph, as before. It is much "hotter" than any carbon we ever had (it has a two-hour half life), and we find out where the oxygen-18 is on the paper. We do not have to know what compounds are present in order to analyze for how much O^{18} is there. We can find out what kind of compound it is from where it is on the chromatogram. We have done a 20-minute photosynthesis experiment in H_2O^{18} as a preliminary trial, and have been able to demonstrate that the O^{18} was where we had expected to find it, primarily in the phosphate area. It will require much further development of this technique to begin to find the early products of water transformation.

There is one last item to describe and that is the photochemical apparatus itself. The photochemical apparatus of the green plants is in the chloroplasts; all the chlorophyll is contained in these chloroplasts. Figure 15 shows an electron micrograph of a very thin section of a chloroplast at a high magnification. On the left is a whole chloroplasts; on the right-hand side it is shown with a still higher magnification, and you can see the layer lattice that forms the grana in the chloroplast.[37] The strata are roughly about 60 A thick, and they are alternating protein- and lipid-type layers.

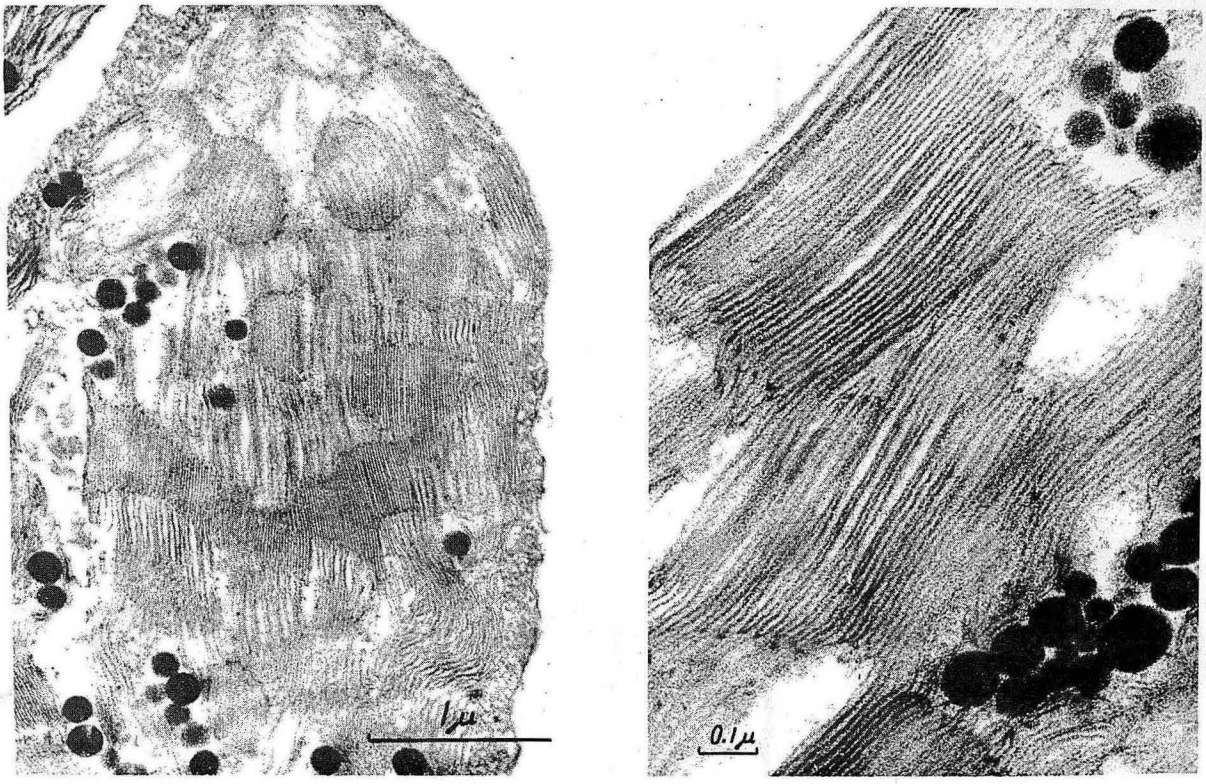


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Fig. 15. Ultrastructure of chloroplasts.

For this and a variety of other reasons, we decided that the chlorophyll (contained in the chloroplast layers) might be functioning photochemically as an ordered array of pigments and associated molecules, and not as individual molecules. The absorption of light in this ordered array raises the electrons from the molecular level to a conduction level. The electrons and holes produced by light absorption are immediately separated to opposite sides of the laminated structure and result in the transformation of absorbed electromagnetic energy in a manner similar to that in a junction photobattery.[38] This function is dependent upon an ordered array of pigments and molecules. This is not photochemistry in solution--it might be called photophysics. There are examples of photoionization of dissolved molecules and there are other types of photochemical reactions of statistically dissolved molecules. I am describing now a photophysical effect, an effect in a quasi-crystalline array of molecules. This idea was first suggested specifically in these terms by Katz in 1949 on the basis of some fluorescence experiments.[39]

There is a more specific physical observation which takes this notion one step further. If the light excites a localized electron from a nonconducting level into what is ultimately a conduction level, by whatever mechanism it may arise, and this electron then wanders around to find an impurity or a defect, and stops there, we should be able to see that odd electron, a trapped electron. Furthermore, the ability to produce such trapped electrons should be relatively temperature-independent. The ability of these electrons to disappear, of course, will depend on the temperature. This we have been able to observe, and Fig. 16 shows what happens.[40,41,42] The electron-spin resonance apparatus detects these odd electrons because of the flipping back and forth of their spin, and we set the apparatus on the peak of the absorption signal and then turn the light on. The signal rises quickly. The rise time is less than a few seconds. When we turn the light off, the signal rise time is practically the same as it is at room temperature, but the decay time is very long, of the order of hours. This rise time is still not an intrinsic rise time of the sample itself. It is simply the rate at which we put photons in. If we put in photons at a high enough rate, we should be able to see the intrinsic rate of transformation, or the rate of capture, of the electrons by the traps, provided the apparatus itself can respond rapidly enough.[43,44]

Figure 17 is a sketch of the entire system, which puts the whole thing together. The sun is in the upper left-hand corner, and in the center is the chlorophyll layer. On one side of it are the holes (+) and on the other, trapped electrons (-). The oxidizing agents leading to oxygen, the reducing agents leading to hydrogen, and the carbon cycle are shown schematically. Carbon dioxide is coming in to the ribulose diphosphate, the C_6 acid, the PGA, etc. Now from this cycle we draw off carbohydrates (which are cellulose and sugars for storage), aromatic compounds, fats, amino acids, proteins, nucleic acids, etc. This gives some idea of the relationship of the various structural features and chemical apparatus of the plant. You can see that we have only just begun the elucidation of the biosynthetic routes to all plant constituents, and we have hardly started the path of oxygen.

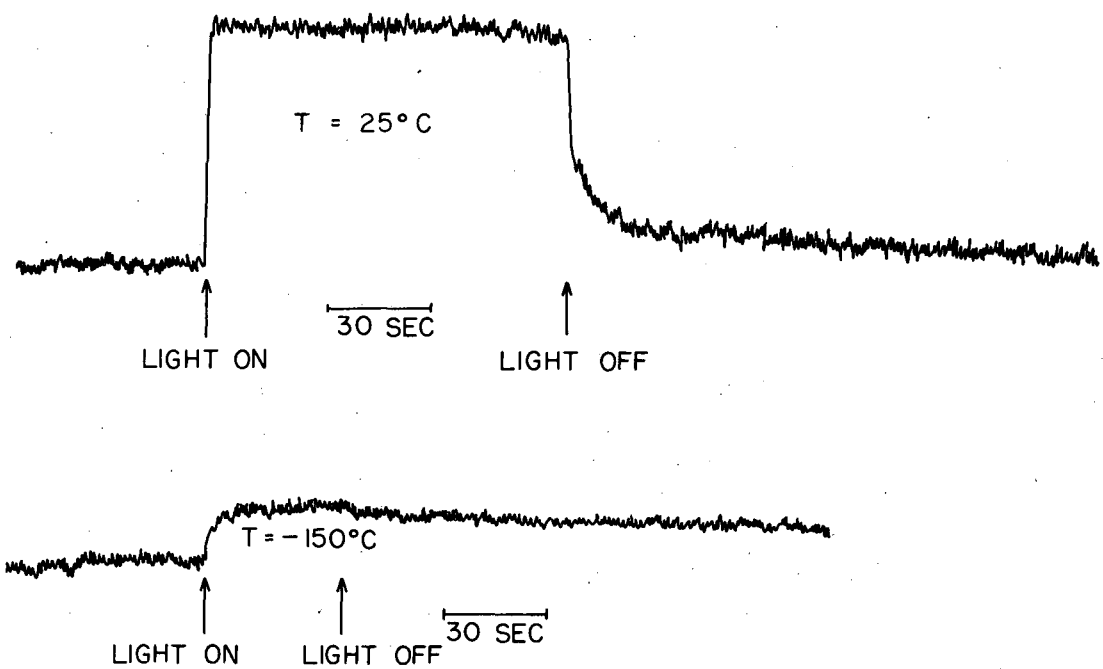

Fig. 16. Signal-growth and - decay time curves of whole spinach chloroplasts at 25°C and -150°C.

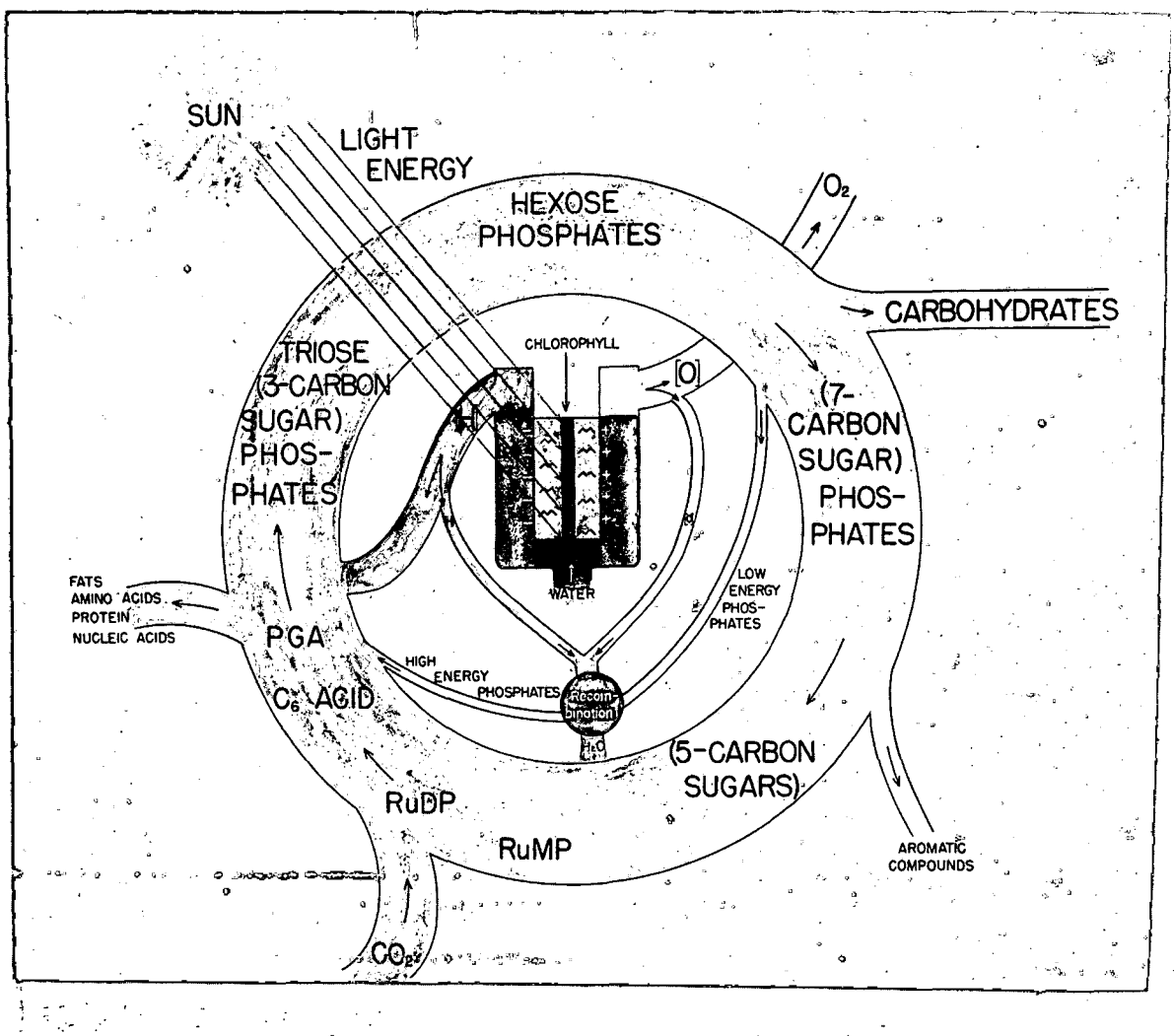

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Fig. 17. Photosynthesis of food from carbon dioxide.

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